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Beneficial Effects of Lysine Acetylsalicylate, a Soluble Salt of Aspirin, on Motor Performance in a Transgenic Model of Amyotrophic Lateral Sclerosis

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We have studied the effect of lysine acetylsalicylate (LAS; Aspegic), a soluble salt of aspirin, on motor deficits in transgenic mice expressing a human superoxide dismutase SOD1 mutation (Gly-93 — Ala), an animal model of familial amyotrophic lateral sclerosis (FALS). In nontreated FALS mice, motor impairments appear at 12–14 weeks of age, whereas paralysis is not observed before 20 weeks of age. Life expectancy is 140–170 days. Early treatment with LAS from 5 weeks of age delayed the appearance of motor deficits in FALS mice as measured by extension reflex, loaded grid, and rotarod tests. This beneficial effect of treatment was maintained up to 18 weeks of age, until just before onset of end-stage disease. When treatment was started at 13 weeks, no significant beneficial effect was observed. These results demonstrate that chronic LAS treatment is able to delay the appearance of reflex, coordination, and muscle strength deficits in this animal model of ALS if the treatment is started early enough. However, neither the onset of paralysis nor end-stage disease were improved by the LAS treatment. In the absence of an effect on survival, the functional improvement demonstrated here is probably the maximum that this demanding model could allow. Although other properties of LAS may have contributed to its beneficial effect, we suggest that the antioxidant properties of aspirin are responsible for the positive effects in this model and support the use of antioxidants as effective therapy for ALS. © 1999 Academic Press

Key Words: transgenic mice; neuroprotection; antioxidant; aspirin; behavior.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder which is characterized by the loss of motor neurons in the cerebral cortex, brain stem, and spinal cord (for review see (9)). The affected neurons demonstrate evidence of cytoskeletal pathology in the form of accumulations of neurofilaments (26). A number of studies suggest that this disease arises from oxidative and excitotoxic injury to critical subcellular targets in motor neurons (6, 8). For example, an 85% increase in protein carbonyl groups, a marker for oxidative damage, has been reported in brain tissue from cases of sporadic ALS (8). In addition, a series of mutations in the CuZn-superoxide dismutase-1 gene (SOD1), located on chromosome 21, have been identified in 15 to 20% of familial ALS (FALS) cases (17, 37), and Wiedau-Pazos *et al.* (42) and Yim *et al.* (44) have demonstrated that oxidative reactions catalyzed by such mutant SOD1 enzymes can initiate the neuropathological changes in FALS.

To test the hypothesis that FALS results from a direct action of the mutant enzyme, transgenic mice that express mutant forms of human SOD1 have been generated (25, 36, 43). These studies demonstrate that mice with a high number of gene copies become paralyzed as a result of motor neuron loss from the spinal cord and die at 5 to 6 months of age. Electromyographic studies have demonstrated that the function of motor neurons in these mice becomes impaired from 7 weeks of age (28), and the onset of motor neuron death is observed from 13 weeks (13). Several studies suggest that neuronal degeneration in these transgenic mice is mediated by free radical production. An increase of lipid peroxidation has been demonstrated in the spinal cord of these transgenic mice (1, 35), and chronic treatment with the antioxidant vitamin E can delay the onset of symptomatology (24).

There are several compelling reasons for testing salicylate and its derivatives in neurodegenerative disorders. First, it has high potency to scavenge hydroxyl radicals as demonstrated by its use in assay systems (14). Such an activity has been suggested *in vivo* in a recent study by Aubin *et al.* (2), who demonstrated the ability of salicylate to block the effects of

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MPTP in mice. Second, salicylate was recently reported to protect against glutamate neurotoxicity via blockade of NF-κB activation (22). Finally, salicylate is readily available and has good brain penetration. Both free radicals and glutamate toxicity have been suggested to play a role in motor neurodegeneration (5, 18, 19). We have therefore studied the effects of a soluble salicylate derivative, lysine acetyl salicylate (LAS, Aspegic), on motor performance and survival of FALS mice. Two treatment regimes were used starting at 5 weeks of age (i.e., before the onset of motor neuron dysfunction) and at 13 weeks of age (i.e., the onset of motor neuron death).

MATERIALS AND METHODS

Transgenic Mice

Transgenic male mice expressing mutant human CuZn-SOD-1 with a $gly^{93} \rightarrow ala$ substitution (designated B6SJL-TgN(SOD1-G93A)1Gur) were bred and maintained by Transgenic Alliance (Saint-Germain-sur l'Arbresle, France). The transgenic mouse line was maintained as hemizygote by mating transgenic males with B6 females. Transgenic progeny were identified by polymerase chain reaction (PCR) amplification of tail DNA (25). Nontransgenic wild-type littermates (wt) were used as controls of FALS transgenic mice. A total of 153 FALS and wt mice were included in two differents experiments (see Table 1). Ninety-seven mice were used for the first experiment in which the effects of LAS on motor deficits and survival were studied; the FALS animals were subdivided in FALSO (no treatment), FALS5 (onset of treatment at 5 weeks of age), and FALS13 (onset of treatment at 13 weeks of age).

TABLE 1

Details of the Experimental Protocols and Numbers of Animals Used in Experiments 1 and 2

Group	Strain	Treatment	Age at start of treatment	Age at start of behavior	Duration testing of treatment	Number tested
			Experiment 1			
FALS0	FALS	Water	—	7	—	18
wt0	wt	Water	—	7	—	13
FALS5	FALS	LAS	5	7	15–19	17
wt5	wt	LAS	5	7	15–19	16
FALS13	FALS	LAS	13	7	7–11	20
wt13	wt	LAS	13	7	7–11	13
			Experiment 2			
—	FALS	water	—	—	—	14
—	wt	water	—	—	—	14
—	FALS	LAS	5	—	3	14
—	wt	LAS	5	—	3	14

Note. Values are expressed as weeks.

Fifty-six mice were used for the second experiment in which brain salicylate levels after the free consumption of LAS solution were evaluated. Mice were housed in groups of 5–7 in macrolon cages (32 × 21 × 14 cm) and kept in an isolation cabinet. They were maintained on a 12-h dark–light cycle (lights on 7:00 a.m.). Throughout the experiment food was placed on the floor of the cage so that mice could easily eat despite appearance of disease symptoms and the water bottle spout was also placed close to the floor. Body weight was measured twice a week.

Treatment Protocol (Table 1)

Experiment 1: Effects of LAS treatment on motor deficits in FALS mice. LAS was dissolved in drinking water at a concentration of 1.8 mg/ml (1.0 mg/ml of aspirin). The 97 male FALS and wt mice were subdivided into three treatment groups: early, in which treatment started at 5 weeks of age; late, in which treatment started at 13 weeks of age; and a control water-treated group. Daily fluid intake of water or LAS solution was estimated by weighing the drinking bottles twice a week, i.e., when the solution was changed (we checked the stability of the salicylate solution up to 1 week after preparation of the solution, as indicated below).

Experiment 2: Salicylate measurements (in the brain and in the consumed water). In order to estimate the stability of the solution, samples were analyzed up to 1 week after the preparation of the solution. To evaluate the passage of salicylate into the brain, 14 FALS and 14 wt mice were given access to the lysine acetylsalicylate solution for 3 weeks (see Table 1 for the treatment schedule). The day before sacrifice, the animals were deprived of drinking water. Three hours before sacrifice, the animals had access to either water or lysine acetylsalicylate. It was necessary to control the period of time preceding sacrifice during which the animals had access to drug solution because brain salicylate was no longer detectable 3 h after an i.p. administration of LAS (unpublished observation). Again, intake of water or LAS solution was estimated by weighing the drink bottles before and after the 3-h period. Finally, the brains were removed and stored at −80°C until analysis. Frozen left hemispheres were sonicated in 500 μl of 0.05 M $HClO_4$ containing 0.5 mM of EDTA (LABOSI) and 2 mM sodium metabisulfite. After centrifugation, the supernatant was diluted in 12 vol of HClO4-EDTA solution and 50 μl were injected onto the liquid chromatography column using a refrigerated (4°C) autoinjector Wisp 712 (Waters, Milford, MA). Separation was achieved at room temperature. The high pressure liquid chromatography (HPLC) system consisted of a pump, a stainless separation column (0.46 × 7 cm) packed with an Ultrasphere XL ODS C18, 3-μm particle size (Beckman, Fullertone, CA). The

mobile phase contained 0.05 M NaH_2PO_4, 2.5 mM octane sulfonic acid, 4% CH_3CN, pH 3. The flow rate was 0.9 ml/min. The detection was carried out by means of a Coulometric detector (model 5100A; ESA, Bedford, MA) equipped with dual analytical cells set at 0.25 and 0.75 V. Concentrations of each compound were calculated using a computing integrator (Maxima, Waters) with reference calibration curves obtained after injection of standards.

Behavioral Testing

The following tests were used to assess motor performance (for more details (4)). All the animals were studied weekly using the loaded grid, rotarod, and extension reflex tests. The first session began at 7 weeks of age. For the Medinaceli test, the animals were studied at 15, 18, and 20 weeks of age.

Loaded grid. The mouse was allowed to grip a small loaded grid weighing either 40, 30, 20, or 10 g and was then lifted by the tail. A maximum period of 30 s was allowed for each weight. The time during which the mouse was able to carry the grid loaded with each of the four weights was measured.

Rotarod. The period for which a mouse could remain on a rotating axle (3.6 cm diameter; speed of rotation, 16 rpm) without falling was measured. The test was stopped after an arbitrary limit of 180 s.

Extension reflex. An extension reflex of the hindlimbs is normally observed when a mouse is suspended in the air by its tail. However, in mice with motor neuron disease, a retraction of the hindlimb is more commonly seen. A score of 2 corresponded to a normal extension reflex of both hindpaws, a score of 1 to the extension reflex of only one hindpaw, and a score of 0 to the absence of any hindlimb extension.

Medinaceli test. Paralysis of hindlimbs was measured in mice using the test described by de Medinaceli *et al.* (16). The hind paws were dipped in ink and the mice were allowed to walk over a strip of paper in a runway (400 × 45 mm). The average stride length was measured for each mouse.

End-Stage Disease

The end-stage criterion corresponds to the motor score 2 defined by Kerasidis *et al.* (29) as "frequent and/or vigorous movement of hindlimbs but no weight bearing."

Statistical Analysis

The data from each experiment were analyzed by the appropriate analysis of variance model. To evaluate the consequence of the SOD mutation on behavior, only the nontreated FALS and wt mice were compared. To estimate the effects of the treatment, only the treated and nontreated FALS mice were compared. Because of the absence of an effect of the LAS treatment on wt mice behaviors, all the wt mice were pooled as the wt group and their behavioral response was shown as the optimal performance for each test. When required, significant interactions were analyzed by Newman–Keuls test. The effect of treatment on survival was evaluated by survival analysis using the LogRank test.

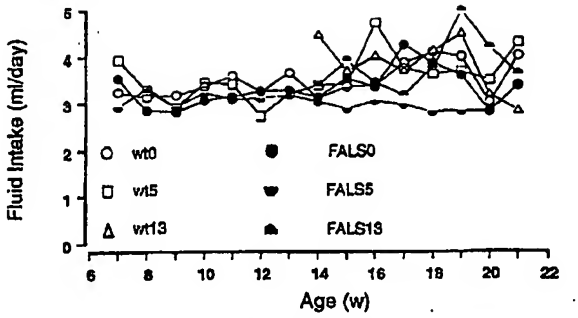

FIG. 1. Daily fluid intake for the LAS- and water-treated wt and FALS mice (experiment 1). Values are expressed as mean daily consumption per animal. See Table 1 for the details of treatment group and for the number of animals per group.

RESULTS

LAS Consumption, Treatment Tolerance, and Salicylate Brain Level Determination

Experiment 1. Daily fluid intake was stable in all groups throughout the experiment (see Fig. 1). Moreover, the presence of LAS and its derivatives did not decrease fluid intake. All the mice drunk approximately 3–4 ml of solution per day, corresponding to an aspirin dose of 3–4 mg/day (approx. 100–160 mg/kg/day). The LAS treatment did not modify body weight, again indicating the absence of toxicity of LAS in our experimental conditions (see Fig. 2).

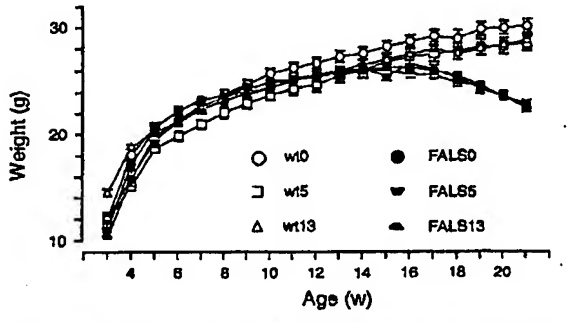

FIG. 2. Evolution of body weight in the experimental groups. Note the absence of deleterious effect of LAS on body weight. Values are expressed as mean and SEM. See Table 1 for the details of treatment group and for the number of animals per group.

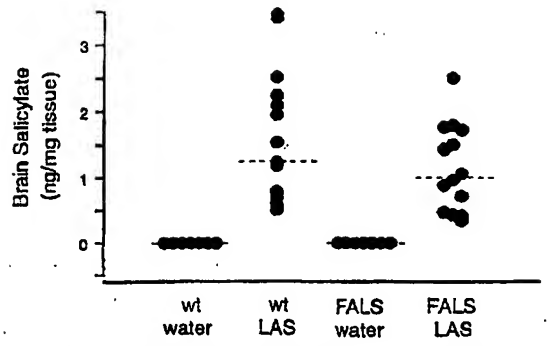

FIG. 3. Brain concentrations of salicylate in treated or nontreated mice (experiment 2). The dashed lines indicate the median value. See Table 1 for the details of treatment group and for the number of animals per group.

Experiment 2. After overnight water deprivation, the mice consumed about 2 ml of solution (2 mg of aspirin) in the 3-h period during which water or treatment was available. Salicylate was detected in the brain of LAS-treated mice at similar concentrations in wt and FALS mice. No salicylate was detected in mice drinking only water (see Fig. 3).

Effects of LAS Treatment on Motor Deficits and Survival

Muscle strength (Fig. 4). Muscular strength was estimated by the loaded grid test. As illustrated in Fig. 4, the young wt mice (7 weeks of age) had more difficulty gripping the heaviest grid than the lightest one (from 40 to 10 g, respectively). As they became older, their performance with the heaviest weights improved. In contrast, FALS mutant mice demonstrated a progressive impairment of muscle strength. There was no difference between the mutant mice and wt mice at 7 weeks of age (ANOVA one-way with group factor: $F(1,29) = 0.59$, n.s.). However, at 15 weeks of age, these two groups differed in their ability to grip the heaviest grids (ANOVA two-ways with group × weight factors interaction: $F(3,87) = 16.77$, $P < 0.001$). From 18 weeks of age, the performance of mutant mice was dramatically impaired whatever the weight of the loaded grid (ANOVA one-way with group factor: $F(1,29) = 107.49$, $P < 0.001$).

The early LAS treatment markedly delayed the appearance of deficits in muscular strength. As shown in Fig. 4, the mutant mice which were treated with LAS from the age of 5 weeks performed better than water-treated FALS mice at 15 and 18 weeks of age (two-way

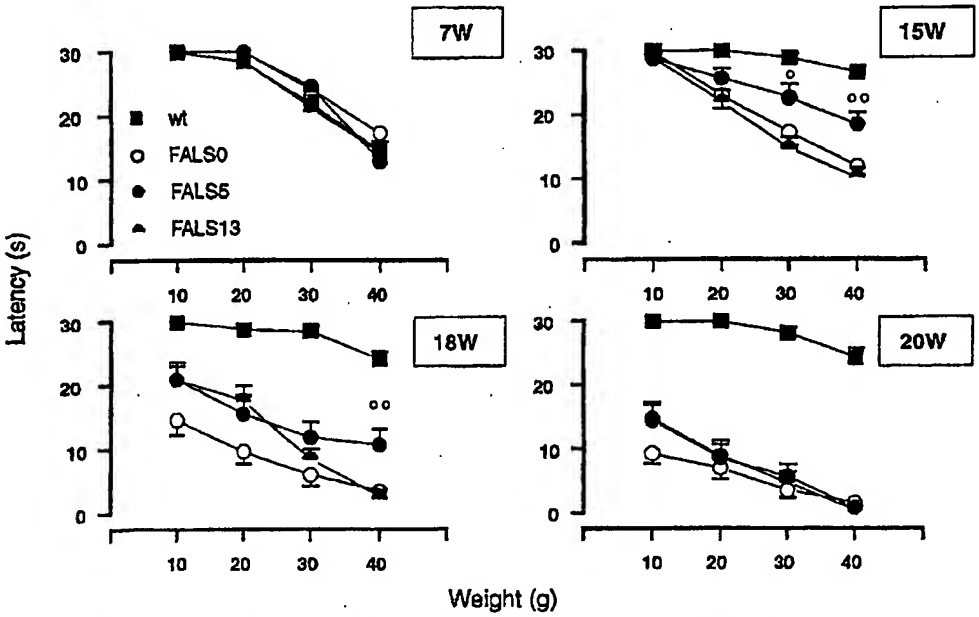

FIG. 4. Muscle strength measured using the loaded grid test at 7, 15, 18, and 20 weeks of age. Note the loss of muscular strength as a function of age in the FALS mice and the beneficial effect of the LAS treatment at 15 and 18 weeks of age. Values are expressed as mean and SEM. See Table 1 for the details of treatment group and for the number of animals per group. Statistical differences between the treated and nontreated FALS mice: oo $P < 0.01$; o $P < 0.05$.

ANOVA weight × group interaction: $F(6,156) = 2.57$, $P = 0.02$; $F(6,156) = 3.54$, $P = 0.003$; respectively). At 20 weeks of age, the two groups were no longer significantly different. The late treatment of LAS did not seem to be beneficial, even if muscle strength had a tendency to be less impaired in the late-treated group than in the water-treated group at 18 weeks of age, as did the reflex from 16 to 18 weeks of age.

Motor coordination (Fig. 5). Motor coordination, estimated by the rotarod test, was also progressively disturbed in FALS mice. As illustrated in Fig. 5, the behavioral performance of the mutant mice decreased from 14 to 21 weeks of age, whereas that of the control wt mice remained stable over the same period (two-way ANOVA: group × age interaction $F(7,203) = 10.46$, $P < 0.001$).

As observed for muscle strength, the early treatment of LAS delayed the appearance of rotarod deficits (two-way ANOVA: group × age interaction $F(14,354) = 3.437$, $P < 0.001$; Fig. 5). The late treatment of LAS had no significant beneficial effect on rotarod performance from 14 to 18 weeks of age, but had a nonsignificant tendency to preserve coordination at 19 weeks of age and after.

Reflex (Fig. 6). The ability of FALS mice to demonstrate the extension of hindlimbs decreased as a function of age: the muscle strength and motor coordination impairments described above were accompanied by a loss of the extension reflex (Fig. 6) starting at 13 weeks of age and which was maximal at 20 weeks of age (two-way ANOVA: group × age interaction $F(14,406) = 31.1$, $P < 0.001$).

The early treatment of LAS delayed the progressive decline of reflex performance [$F(28,728) = 2.411$, $P < 0.001$]. This beneficial effect was more marked than those evaluated on muscle strength and motor coordination, but the decline in performance of treated FALS mice was rapid and sudden after 18 weeks of age.

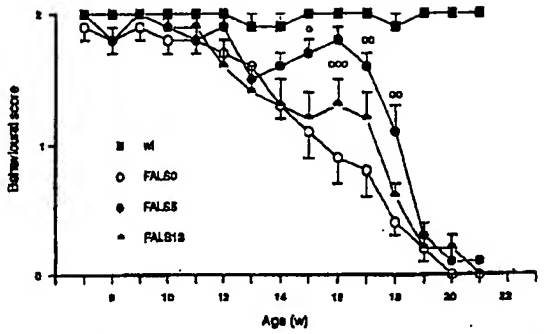

FIG. 6. Evaluation of the extension reflex in the different experimental groups. Note the progressive loss of reflex as a function of age in the FALS mice and the beneficial effect of the LAS treatment. Values are expressed as mean and SEM. See Table 1 for the details of treatment group and for the number of animals per group. Statistical differences between the treated and nontreated FALS mice: ooo $P < 0.001$; oo $P < 0.01$; o $P < 0.05$.

Stride length (Fig. 7). Unlike the behavioral performances described above, impairment of the stride length did not appear until very late, at 20 weeks of age (two-way ANOVA: group × age interaction $F(2,58) = 27.22$, $P < 0.001$). Treatment did not prevent the stride shortening (two-way ANOVA: group × age interaction $F(4,104) = 1.213$, n.s.).

Survival (Fig. 8). In FALS mice, the end-stage criterion was reached at 140–170 days of age and LAS treatment had no beneficial effect on this measure (Log-Rank test, Chi-Square = 3.88, $df = 2$, n.s.).

DISCUSSION

The aim of the present study was to evaluate potential beneficial effects of lysine acetylsalicylate in a

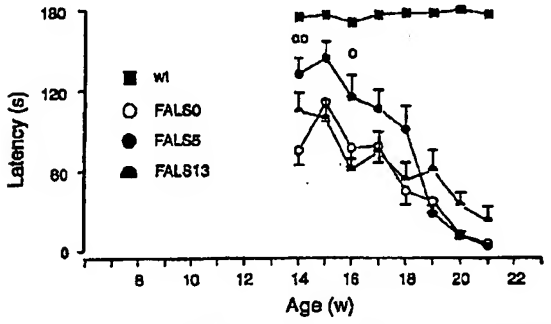

FIG. 5. Motor coordination of experimental groups measured using the rotarod test. Note the appearance of motor incoordination as a function of age in the FALS mice and the beneficial effect of the LAS treatment. Values are expressed as mean and SEM. See Table 1 for the details of treatment group and for the number of animals per group. Statistical differences between the treated and nontreated FALS mice: oo $P < 0.01$; o $P < 0.05$.

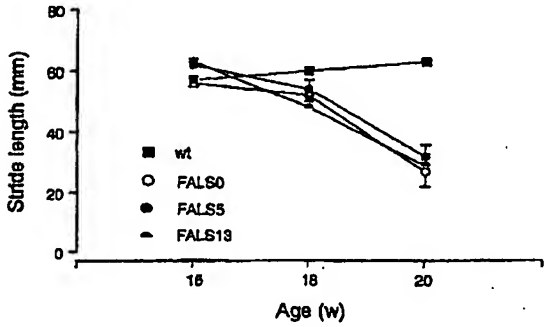

FIG. 7. Stride length of the experimental groups measured with the Medinaceli test. Note the shortening of stride length at 20 weeks of age in the FALS mice and the absence of beneficial effect of LAS treatment. Values are expressed as mean and SEM. See Table 1 for the details of treatment group and for the number of animals per group.

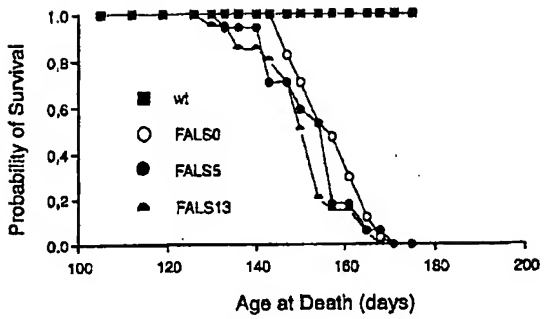

FIG. 8. Survival of FALS mice. Note that LAS treatment has no effect on survival (measured as appearance of end-stage criterion). See Table 1 for the details of treatment group and for the number of animals per group.

model of ALS. Chronic LAS treatment delayed the appearance of motor deficits in FALS mice when the treatment was started early enough. This beneficial effect of treatment was maintained for 6 weeks, until 2 weeks before onset of end-stage criterion. However, LAS treatment had no effect on onset of end-stage disease.

Bioavailability and Tolerance of LAS Treatment

Because mice were administered LAS in drinking water, it was first necessary to evaluate the effect of salicylate on fluid consumption and to verify the ability of salicylate to cross the blood–brain barrier equally in wt and FALS mice. Lysine acetylsalicylate was used in our experiments because of its high solubility in water: immediately after dissolving, LAS is hydrolyzed to aspirin and then to salicylate, which is highly stable in solution. Indeed, we confirmed an unchanged concentration of salicylate in the water bottle up to 1 week after the preparation of the solution (data not shown).

The presence of salicylate and derivatives did not modify fluid consumption of the experimental groups. In addition, the volume of consumed solution (water with or without LAS) remained stable throughout the experiment; i.e., mutant FALS mice exhibiting motor deficits continued to receive the same quantity of aspirin as control wt mice. Our results are in accordance with those of Gurney (23) who reported stable food consumption during aging in mutant FALS mice. The LAS dose consumed by the animals was about 180–300 mg/kg/24 h (aspirin dose: 100–160 mg/kg/24 h), which is lower than the LAS dose of 500 mg/kg/24 h, at which side-effects appear (gastric lesion, body weight decrease, data on file, Synthelabo Research). This is supported by the observation that LAS treatment did not change the evolution of body weight of the mice in our experiment.

Finally, we confirmed the presence of salicylate in the brain of mice after they were administered LAS in drinking water. The amount of salicylate found in the brain was 3 times less than that measured one and a half hours after an i.p. injection of 100 mg/kg aspirin (2). In conclusion, the LAS treatment procedure used in our experiment was suitable for studying the effects of LAS on the progressive motor deficit and on survival in FALS mutant mice.

Progression of Motor Deficits in FALS Mice

The FALS mice have been well characterized in both electrophysiological (3, 28) and histological (13) studies. Motor neuron function starts to become impaired at 7 weeks of age and, in parallel with a reduction in motor unit number, the remaining functional units show an increase in number of neuromuscular junctions compared to controls at 8–9 weeks. The onset of cell death was observed from 13 weeks. We have previously reported some parallels between the time of appearance of these pathologies and the progression of motor deficits in FALS mice (4). Here, we confirmed the motor impairments for certain behavioral measures. For example, extension reflex and motor coordination were impaired from 12 and 14 weeks of age, respectively. Muscle strength of FALS mice, evaluated in the loaded grid task, was also impaired from the age of 13 weeks (results not shown), and in the present studies we evaluated the strength of the animals in more detail. Transgenic mice exhibited mild impairments which depended on the weights of the loaded grid. For example, at 15 weeks of age, FALS mice exhibited poor performance with the heaviest weights, whereas normal performance could be observed for the lightest weight. Finally, we studied the onset of the hindlimb paralysis by measuring the stride length in the Medinaceli task. Unlike the other motor behaviors previously described, paralysis appeared late (after 18 weeks of age) but developed rapidly (50% deficit in 2 weeks). Such a late, but fast, evolution has also been reported by Chiu *et al.* (13) for the same task, although the onset of paralysis was later in our experiment than in those of Chiu (140 and 125 days, respectively). The onset of end-stage criterion reported here was also delayed by about 20 days as compared to that previously observed by us (4) or Chiu *et al.* (13), i.e., 155 days versus 140 and 136 days, respectively. One explanation may be found in a small shift of the G1H genotype. Indeed, the G1H line was identified as a spontaneous expansion in transgene copy number of 40% over the original G1 line described in Gurney *et al.* (25) and thus may be subject to a partial loss of the extra gene copies.

LAS Delays the Appearance of Motor Deficits

The early treatment of LAS delayed the impairment of reflex, muscle strength, and motor coordination in

FALS mice measured by the extension reflex, the loaded grid, and the rotarod, respectively. This motor improvement could be observed up to 18 weeks of age and was particularly marked for the extension reflex. The late treatment was not as efficacious as the early treatment, although there was a tendency for muscle strength and reflex to be less impaired in the late-tested group at 18 weeks of age, or from 16 to 18 weeks of age, respectively. The fact that the treatment which preceded the onset of neuronal dysfunction (i.e., starting at 5 weeks of age) was more efficacious than the one occuring just at the onset of neuronal death (i.e., starting at 13 weeks of age) suggests a neuroprotective effect of LAS. However, the motor performance of treated FALS mice was as poor as that of nontreated mutant mice at 20 weeks of age, and LAS treatment was unable to delay the onset of end-stage disease. The fact that LAS treatment was ineffective on the progression of paralysis was probably because paralysis onset occurred just before death. Indeed, no gait amelioration could be observed at 15 and 18 weeks of age because no clear shortening of stride length could be observed in these ages, and no amelioration was observed at 20 weeks. The early LAS treatment was thus able to delay motor deficits up to two weeks before end-stage disease, which seems to be the maximum effect we can expect for a compound which had no effect on survival.

These results indicate that measures of functional motor progression and survival are separable in the transgenic model and may be affected somewhat independently by therapeutic agents. This idea has already been formulated by Gurney *et al.* (24), who demonstrated differential effects of two compounds in FALS mice: dietary supplementation of the antioxidant vitamin E delayed onset of clinical symptoms but did not prolong survival, whereas riluzole, which interferes with glutamatergic neurotransmission, prolonged survival without any affect on disease onset.

Currently, at least three pathogenic pathways have been proposed to account for ALS: cytoskeletal abnormalities such as excessive accumulation of neurofilaments (15); excitotoxicity resulting from the loss of glutamate transporters (39) and chronic activation of glutamate receptor subtypes (38); and oxidative stress (42). Trophic factors have been the subject of intense interest in recent years with regard to their ability to protect against motor neuron degeneration and CNTF, BDNF, IGF-1, and GDNF have been studied extensively in mouse models of motor neuron degeneration, including the wobbler mouse (34) and the progressive motor neuronopathy (pmn) mouse (40, 41). Although CNTF has been shown to be neuroprotective in the two animal lines, it was completely ineffective in two large clinical trials of ALS patients, probably because of its high toxicity (11, 12). This suggests that, although CNTF was effective in the wobbler and pmn models, the models themselves fail to mimic an important component of the degenerative process in ALS, and thus may not be good as preclinical screening tools. Transgenic FALS mice carrying a SOD1 mutation known to produce the disease in man closely mimic the human pathology and probably represent the best animal model for studying novel therapies for ALS. Indeed, riluzole, which has beneficial effects in humans (7, 32), is also effective in the FALS mouse model. As in humans, CNTF was unable to improve clinical symptoms and survival of transgenic SOD mice. Therefore, compared to other models (mutant mice, facial nucleus lesion) of motor neuron degeneration, the SOD transgenic mouse appears to be a demanding, but perhaps more appropriate, model of ALS for the discovery of promising therapeutic agents such as LAS.

Our results demonstrate that onset of illness (measured by muscular strength, motor coordination, and reflex) was delayed for 2–3 weeks (10–15% of life expectancy) when LAS treatment started at 5 weeks of age. This beneficial effect compares favorably with other results. The overexpression of bcl-2 in FALS mice both delayed onset of illness by 15% and prolonged survival by 12.5% (30), whereas inhibition of interleukine-1β-converting enzyme (ICE) by site-directed mutagenesis prolonged survival by only 8% (20). In the case of pharmacological treatments, riluzole is reported to prolong survival by 8% (24), vitamin E to delay onset of motor neuron disease by 8% (24), and *d*-penicillamine to prolong survival by 10% (27). Comparison between our results and those published by these four teams indicates that the functional improvement in motor performance that we obtained after LAS treatment is among the best thus far demonstrated in this animal model.

Mechanism of Action

The mechanism(s) by which LAS ameliorated motor performance in FALS mice is (are) not yet fully understood. Aspirin is well known for its analgesic, anti-inflammatory, and anticoagulant properties, mediated via inhibition of prostaglandin synthesis (33). Another property of salicylate is its high potency to react with hydroxyl radicals and to be converted to 2,3- and 2,5-dihydroxybenzoic acid (14). We support a neuroprotective role for aspirin, salicylate, and LAS activity in another *in vivo* model of neurodegeneration based on oxidative dysfunction: salicylate, aspirin, and LAS prevent the neurotoxic effects of MPTP in mice (2), a neurotoxin which acts through elevated production of superoxide and peroxynitrite radicals and which is blocked by NO-synthase inhibitors. Wiedau-Pazos *et al.* (42) demonstrated that the SOD mutants A4V and G93A were able to potentiate the oxidative properties of hydrogen peroxide and to induce elevated production of hydroxyl radicals. This enzymatic reaction is copper-

dependent, and a treatment with the copper chelator penicillamine increases survival of FALS mice (27). If motor neuron degeneration in FALS mice is mediated by free radicals, hydroxyl radical scavengers such as aspirin would be expected to act like penicillamine and delay the onset of end-stage disease. We have no explanation as to why LAS failed to delay the onset of end-stage criterion, whereas it considerably delayed the appearance of motor deficits. It is possible that these two antioxidant compounds interact with different cellular compartments or at different levels of the process of neuronal death. Another possibility is that scavenging hydroxyl radicals is not the main mechanism by which aspirin has beneficial effects on motor functions in FALS mice. A recently discovered property of aspirin has been reported by Kwon *et al.* (31), who demonstrated that aspirin inhibits inducible NO synthase and NO production. Moreover, another recent paper (10) questions the presence of elevated concentrations of hydroxyl radicals in the spinal cord of another transgenic FALS strain (43), but suggest instead a role for tyrosine nitration in neurodegenerative events through the peroxynitrite radical. Peroxynitrite radical formation results from the reaction between the superoxide radical O_2^- and the nitrogen monoxide, and SOD mutants have been shown to increase superoxide production in PC12 cells (21). Aspirin may therefore ameliorate motor performance in FALS mice by diminishing the formation of the peroxynitrite radical.

In conclusion, our results demonstrate that the soluble aspirin, i.e., LAS, is able to retard the onset of deficits of coordination of movement, muscle strength, and reflex in this transgenic animal model of ALS. To our knowledge, this functional improvement in motor performance is the most marked thus far demonstrated in this animal model. Whereas we cannot exclude the possibility that the other properties of aspirin may have contributed to the beneficial effect, our results using transgenic SOD mice reinforce the possibility that aspirin may protect from neurodegeneration through antioxidant properties.

There is a clear need for effective treatment of ALS and, to date, riluzole is the only therapeutic compound available. As riluzole seems to prolong survival in humans without affecting onset, cotreatment with a compound which delays onset of motor symptoms, such as aspirin, would be of great value. One of the aims of our work is to communicate to physicians the potential therapeutic usefulness of aspirin, a generic, harmless, and well known drug, in ALS. In particular, early treatment with aspirin (i.e., starting before the onset of symptoms) may be of great benefit in patients with a familial form of ALS.

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